results upon binding to the closely related UNC5A, UNC5B, UNC5C or UNC5D receptors. In addition to the nervous system, netrin-1 and its receptors have been detected in developing heart, lung, mammary gland, intestine and pancreas and are believed to be expressed ubiquitously. Both families of receptors have been implicated as dependence receptors, that is receptors that induce apoptosis if they are not bound to their ligand such as netrin-1. Thus they play a pivotal role in the development of the organism and in tumour suppression.

Here, we present the 2.5 Å high resolution structure determined by X-ray crystallography that comprises four domains of netrin-1. We mapped the netrin-1/neogenin and the netrin-1/UNC5 binding sites by a series of domain swap experiments where we exchanged individual domains of netrin-1 with a structurally closely related -but non-functional- domain of the laminin γ1 short arm. We probed the binding behaviour of the chimeras by surface plasmon resonance and solid phase binding assays. Once the binding domain had been unambiguously identified, we determined the binding epitopes on netrin-1 by mutational analysis, using structural information and phylogenetic conservation as guides. We found that the DCC family and the UNC5 family of receptors bind to spatially distinct sites within the same domain. Reciprocally, we confirmed that the 5th fibronectin domain of DCC harbours the binding site for netrin-1 and determined the binding epitopes. Finally, we show the conformation of the netrin-1/neogenin complex in solution using SAXS data and present a map of the binding sites.

2420-Pos Board B112

Molecular Interaction Between PPTI and Shakv1.1 Potassium Channel Explored by Docking and Molecular Dynamics Simulation

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PPTI, proposed as a potential dendrotoxin, which isolated from the venom of Pseudocerastes persicus has 58 amino acid residues and cross-linked by three disulfide bonds. We explored the interaction between PPTI and ShaKv1.1 potassium channel by successive application of protein-protein docking and molecular dynamic simulation. The analysis of the complexes revealed that the Lys5 residue of PPTI plugged its side chain into the channel selectivity filter. Molecular dynamic simulation in membrane environment of the complex structure resulted in docking stage confirmed the stability of the complex.

2421-Pos Board B113

Elucidating Time Course of Structural Changes Leading to Receptor-Ligand Complex Formation with Transient-EPR

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Protein functions rely on protein motions. Protein structures, obtained with X-ray diffraction or NMR, reveal mostly static pictures of structure-function relation. To connect structural changes of proteins with function, we need to look at events happening in real time, i.e. the dynamics of the processes.

We have chosen mlCNBD as a model protein to study the dynamics of receptor-ligand complex formation. mlCNBD is a cytosolic cyclic nucleotide-binding domain of a bacterial potassium channel (MloK1). mlCNBD binds to cyclic adenosine monophosphate (cAMP) and undergoes conformational changes as evident from the NMR and X-ray structures of the *apo* and *holo* conformational states of the protein. Recent kinetic and NMR studies indicate that these structural transitions follow the "induced-fit" mechanism, i.e. these are a direct consequence of ligand binding. However, the detailed mechanism of these structural rearrangements leading to receptor activation remains elusive.

We use transient Electron Paramagnetic Resonance (tr-EPR) spectroscopy in conjunction with Site- Directed Spin Labelling (SDSL) to resolve the dynamics of mlCNBD-cAMP complex formation. We introduce single cysteine residues at different sites in the protein. The mutants are labelled with Methane Thio Sulphonate Spin Label (MTSSL). Binding of cAMP to the mutants is rapidly initiated either via a caged-cAMP approach or through a micro-mixer. The time-resolved EPR data reveals the progression of structural changes taking place at a particular site on millisecond time scale. Collating data across the whole protein will enable us to reconstruct the steps from the *apo* to the *holo* state of the protein. It will also provide the answer to the question whether the "induced-fit" mechanism follows a concerted (single step) or sequential (multi-step) path from the *apo* to the *holo* conformation.

2422-Pos Board B114

Exploring the Binding Site of the G Protein-Coupled Receptor GPR119 Model using a Pair of Diastereomers with Opposing Action

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GPR119 is a Class A (rhodopsin-like) G-protein coupled receptor that has insulin-regulating activity. GPR119 agonists stimulate glucose-dependent insulin secretion in vitro and lower elevated blood glucose in vivo. Hence, the GPR119 receptor has emerged as an important target for the treatment of type 2 diabetes. An initial GPR119 homology model was constructed using the adenosine A2A receptor crystal structure bound to an antagonist at 2.6Å resolution (PDB id: 3EML) as the template. The preliminary model was refined by exploring the flexibility of individual helices using the Monte Carlo/simulated annealing method, Conformational Memories (CM). CM employs multiple Monte Carlo/simulated annealing random walks, exploring peptide and protein dihedral conformational space. A pair of previously reported diastereomers, one of which acts as a GPR119 receptor agonist and the other as an antagonist, were docked into the active (R*) and inactive (R) receptor models. The putative binding site of the preliminary R model starts between the top of transmembrane helices (TMHs) 2 and 7 and spans the bundle in a shallow diagonal direction, almost perpendicular to the TMH region. The floor of the binding site is between TMHs 3, 5 and 6 at the level of F234(6.44) (middle of the TMH bundle). W238(6.48) ($\chi1 = g+$) places its bulk between TMHs 6 and 7. The only charged amino acid that is accessible to the binding site, R81(3.28), was used as the primary interaction site for both compounds. The GPR119 R* state binding site is more vertical compared to the R state and somewhat deeper because of a $\chi1$ rotamer change of W238(6.48) to a trans conformation in the R* state, which places its bulk between TMHs 5 and 6.

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A Comparison of 3D Conformations of Endothelin-1 Analogs to Find the Pharmacophore Model Required for Endothelin Receptor Ligand Activity

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Endothelin receptors (ETRs) are GPCRs whose activation results in the increase of intracellular calcium levels, and are ultimately involved in vasoconstriction and vasodilation. Because control of ETR activity has important medical implications, it is beneficial to rapidly discover effective synthetic ETR ligands by virtual drug screening methods. Despite its biological importance, little is known about ETR structure, since there are no reported X-ray structures to date. However, structural information is available for Endothelin-1, the natural ligand to ETR and the most potent vasoconstrictor yet identified. Thus, our work takes the ligand-based approach instead of receptor-based approach to ETR ligand pharmacophore discovery. Two well-studied ETR ligands, BQ-123 and bosentan, were chosen to be reference compounds in addition to Endothelin-1, and 30 known synthetic ligands were defined as the sample active compounds set. Conformers of the sample set were prepared by genetic algorithm-based approach using OpenBabel, while conformers of the reference set were prepared by molecular dynamics, using myPresto/cosgene. For molecular superimpositions, we built and used a program called 'BMSIP,' which is an algorithmic extension of the ROCS program. While it performs rigid-body superimpositions using a volume overlap score function based on spherical Gaussian descriptions of atomic shape, BMSIP also introduces a scores-weighting matrix to bias atom-pairings by atom-type correspondence. Superimposition of the sample set against the reference set was performed to obtain a density map that shows the frequency of atom overlaps between the superimposed compounds and reference compounds. This map can indicate the essential chemical features (the pharmacophore) required for interaction with ETRs. The results suggest that nonpolar groups alone, in particular aromatic groups, are important for ETR binding affinity and provide a good initial guess for directed ETR ligand design.